TOPIC 19 – Heart failure, cardiomyopathies – A

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Cardiac infiltration is an independent risk factor of survival after liver transplantation in transthyretin amyloidosis

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Background: Hereditary transthyretin amyloidosis (HA-TTR) is an autosomic dominant disease caused by mutated TTR, leading to both polyneuropathy and infiltrative cardiomyopathy. By removing the main source of mutated TTR, liver transplantation (LT) improves patient's prognosis. Our aim was to document the influence of cardiac and neurological status on survival after LT.

Method: In HA-TTR patients having been liver-transplanted since 1993 in our French reference center, survival was analyzed by using a multivariate Cox model including age, sex, mutation type and preoperative characteristics including neurological Norris score, relative wall thickness (RWT) measured by echocardiography and invasive intraventricular pressures. ROC curves (5-year survival) were also obtained.

Results: The present interim analysis included 155 HA-TTR patients (46±13 yrs, 67% Val30Met mutation). During a mean follow-up of 77±56 months, 43 patients died (1-year survival 92±2%, 5-year survival 79±4%, 10-year survival 66±5%). Non survivors were older, mostly males, with less frequent Val30Met mutation, lower Norris score, thicker cardiac walls and higher LV and RV pressures. Multivariate analysis indicated that RWT and Norris score were independent predictors of death (RWT; HR=87. 95%IC: 8-900, p=0.0002; Norris score: HR=0.94, 95%IC: 0.9-0.98, p=0.002) while age, sex and mutation type were not. The 5-year survival following LT was 23±12% for patients with RWT>0.526 and Norris score <86% vs 98±2% for patients with RWT<0.526 and Norris score >86% (log rank test p<0.0001).

Conclusion: Cardiac infiltration and the severity of polyneuropathy were independent risk factors of poor survival in liver-transplanted patients with HA-TTR.

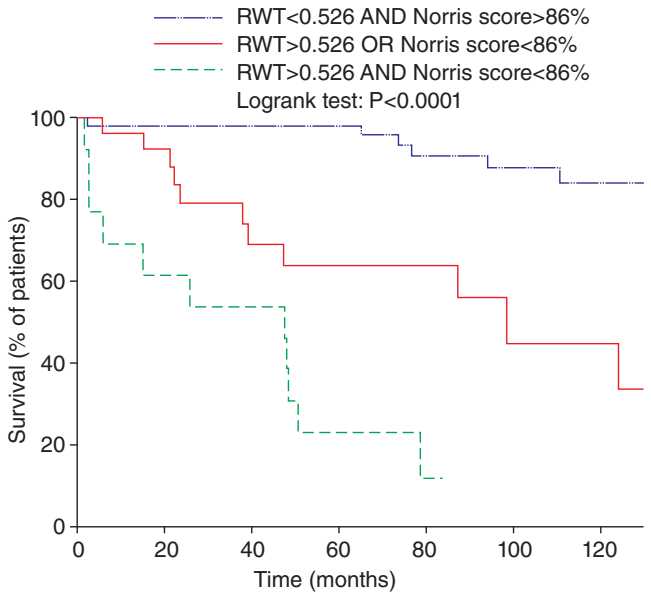

Figure – Abstract 269 – Risk factors and survival after LT in FAP patients.

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Protective effect of saffron extracts against doxorubicin cardiotoxicity

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Doxorubicin (DOX) continues to be used extensively to treat various malignancies, yet the usefulness of this drug is limited by its cardiotoxicity. Subcellular changes leading to this toxicity are suggested to be mediated through an increase in free radicals and lipid peroxidation.

The aim of the present study was to assess whether a 6 weeks supplement with saffron (SAF), a natural antioxidant, in rabbits might prevent the cardiotoxicity of DOX treatment. 40 adult male rabbits (Oryctolagus *cunnilicus*) were used in this study.

They were randomized into 4 groups (n=10 /group). Group I (control) without any treatment. Group II received orally an optimal dose of saffron extracts (5 mg/kg bw) daily for 6 weeks. Group III received a single IP dose of DOX weekly for 4 weeks at a dose of (2.5 mg/kg bw) starting from the 3rd week. Group IV received SAF as in group II and DOX injection as in group III. One week after the end of DOX treatment, the surviving rabbits were sacrificed and their hearts were perfused on a Langendorff mode and subjected to a 30 min period of global ischemia followed by 30 min reperfusion.

The results were as follows: saffron supplementation in DOX treated rabbits significantly 1) reduced mortality (3 in DOX group III and 1 in group IV); 2) improved cardiac function recovery and reduced the incidence of arrhythmias at reperfusion; 3) reduced infarct side; 4) increased the activity of myocardial glutathione peroxidase (GPx) and reduced malondialdehyde content; 5) reduced damage and necrosis of cardiomyocytes after histopathological examination.

In conclusion, this study demonstrates that saffron supplementation is able to limit DOX cardiotoxicity in isolated rat hearts submitted to a sequence of ischemia/reperfusion.

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Prevalence and severity of sleep apnoea syndromes in cardiac amyloidosis patients

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Background: Cardiac diseases are associated with a high prevalence of sleep apnoea syndrome (SAS) particularly in heart failure. Two types of SAS are known: central or obstructive. Heart failure can occur in patients with primary systemic amyloidosis (AL), senile systemic amyloidosis (SSA), and Transthyretin-Related Amyloidosis (TTR). There is no data about prevalence and severity of sleep disordered breathing in cardiac amyloidosis.

Aims: To assess the prevalence and severity of SAS in cardiac amyloidosis.

Methods: Patients prospectively referred in our cardiology department for cardiac amyloidosis underwent polygraphy to diagnose sleep apnoea syndrome (SAS) between 2010 and 2012. SAS was defined as an apnoea-hypopnoea index greater or equal to 5 events/h.

Results: Thirty five patients were included, of whom 15 had AL, 9 FAP and 11 SSA. Mean age, left ventricular ejection fraction, and body mass index of the overall cohort were respectively 75±12 years, 45±13% and 24±4 kg/m². The prevalence of SAS was 86%. 29% of syndromes were classified as central and 57% as obstructive. The mean apnoea hypopnoea index was 22±14 events/h and was superior to 30 events/h in 11 patients. AHI was more elevated in SSA and FAP than in senile. NTproBNP was not different between the three types of amyloidosis.

Conclusion: The prevalence of sleep-disordered breathing is high in cardiac amyloidosis population, with most syndromes having an obstructive pattern. Effect of SAS treatment should be investigated in this population.

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Iron overload does not potentiate doxorubicin cardiotoxicity in mice

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Background: Doxorubicin (DOX), an anthracycline used as an anticancer drug, is known to cause potentially life-threatening cardiotoxicity. Most of the proposed mechanisms involve oxidative stress. DOX forms a stable complex with iron. The mechanisms of interactions between iron metabolism and DOX-induced cardiotoxicity remain a matter of controversy.

Objectives: The role of iron in DOX cardiotoxicity was studied using a murine model of iron overloading (IO). We evaluated cardiac functional parameters, nitro-oxidative stress levels and iron status.

Methods: For 3 weeks male C57BL/6 mice received a daily intraperitoneal injection of dextran iron (15 mg/kg/day) (n=16) or saline injection (n=16) and were then injected with a single dose of 6 mg/kg DOX or saline (8 animals in each group). Cardiotoxicity was assessed using echocardiography, myocardial expression of natriuretic peptides (ANP, BNP) and beta-myosin heavy chain. Oxidative stress was assessed by electron spin resonance spectroscopy (ESR) using a spin probe (CMH hydroxylamine).Iron content was evaluated by atomic absorption spectrometry.

Results: At the end of the treatments, there was a significant fall in left ventricular ejection fraction (LVEF) in animals treated with DOX alone (DOX group) and in animals with prior IO treated with DOX (IRON-DOX group) as compared to their baseline values. LVEF impairment in IRON-DOX mice was similar to that in DOX mice (51±5% vs 44±7%). Unlike the DOX group, there was no increase in left ventricular diameter and ANP mRNA cardiac levels compared to baseline values in IRON-DOX group. However, the IRON-DOX group had a significantly higher myocardial expression of BNP than the DOX group. IO alone (IRON) resulted in cardiac hypertrophy as assessed by an increase in LV weight (+25%). The CP• signal intensity was significantly higher in the IRON group than in the control group, and higher in the IRON-DOX group than in the DOX group.

Conclusions: IO did not result in a significant increase in DOX cardiotoxicity in mice. Conversely, our results suggest the role of prior chronic oxidative stress induced by IO could mitigate acute DOX cardiotoxicity.

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Effects at 12 months of alcohol septal ablation on diastolic dysfunction in patients with hypertrophic obstructive cardiomyopathy

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Background: Alcohol septal ablation (ASA) is an alternative therapy to septal myomectomy or dual chamber pacemaker for treatment of symptomatic hypertrophic obstructive cardiomyopathy (HOCM). Efficiency of ASA on left ventricle (LV) diastolic dysfunction, a major determinant of symptoms and outcome, has been poorly described until now.

Objective: To evaluate the procedural and one-year clinical and echocardiographic outcomes, with a specific focus on diastolic dysfunction, in patients with HOCM treated by ASA.

Methods: A prospective observational study was conducted in a single institution (Institute of Cardiology, Pitié Salpêtriere hospital, Paris) for consecutive patients undergoing ASA. All patients had clinical (NYHA class, cardiovascular event) biological (BNP) and echographic monitoring during the follow-up (3, 6 and 12 months). Data were analysed using the paired t test (α=0.05).

Results: 23 patients were included (12 men and 11 women) with a mean age of 60 y. For all patients, a single septal branch was treated using a total of 1 to 2.5 mL of ethanol (mean 1.80) in 2 to 5 injections (mean 3.80). Results of BNP, E/A, E/Ea, DT and LVOT (LV outflow tract) were summarize in Table 1. There was a significant decrease of LVOT gradient (58.4 to 34.9, $p<0.01$) and inducible LVOT gradient (123.7 to 97.7 mmHg, $p<0.01$) at 12th month. Three months after ablation, E/A ratio decreased from 1.51 to 1.26 ($p=0.007$). There was a significant decrease of BNP (1046 vs 314 ng/l at 6 months, $p=0.004$) resulting in a clinical improvement (median NYHA at baseline was 3, median NYHA at 3, 6 and 12 months was 2). 3 complications (ventricular arrhythmias, aortic valve replacement for shredding of aortic cups, Scarpa hematoma) occurred on peri-procedural survey and 2 non-cardiac deaths (mesenteric ischemia and ischemic stroke) occurred during the follow-up.

Conclusion: ASA appears as an effective treatment of HOCM at 12 months, regarding effect on obstruction but also on LV diastolic function.

Table 1 – Abstract 400 – Evolution of BNP and echographic parameters from baseline to 12 months

	Baseline	3 months	6 months	12 months
BNP, mean	1046	642	314*	443
Basal LVOTG gradient, mean	62.0	34.1*	37.3*	34.9*
Inducible LVTOG, mean	129.1	87.6*	72.5*	97.7*
E/A, mean	1.54	1.26*	1.41	1.45
E/Ea, mean	10.11	9.18	9.15	7.41
DT, mean	225	254	232	217

Footnotes: *values with significant difference ($p<0.05$) with baseline value. BNP = B-type natriuretic peptide. LVOTG = Left ventricular outflow tract gradient.

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Doxorubicin cardiotoxicity: Altered calcium signaling in doxorubicin treated mice

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Doxorubicin (Dox) is a highly effective cancer chemotherapeutic agent, but its clinical usefulness is limited because of its dose-dependent cardiotoxicity. Dox treatment can cause severe cardiomyopathy, as left ventricular dysfunction thus leading to fatal congestive heart failure (HF).

Among the pathophysiological mechanisms underlying dox-induced cardiotoxicity, dysregulations of calcium homeostasis and remodeling of the β-adrenergic signaling are involved but still need further investigation. Epac (exchange protein directly activated by cyclic AMP) could play an important role in the progression of dox-induced HF in regard to its signaling and recent role in cardiac remodeling.

Therefore, we investigated Ca^{2+} handling in a mice model treated with a total accumulated dose of 12 mg/kg of dox, 2, 6 and 15 weeks after last intravenous injection using patch-clamp, confocal imaging and western blot. Echocardiography of dox-treated mice showed a depressed contractile function at 15 weeks, correlated with a reduced and slower intracellular Ca^{2+} transients, increased incidence of Ca^{2+} sparks, and no significant differences of the action potential duration and membrane capacitances. At the molecular level, among the proteins analyzed involved in excitation-contraction coupling, we observed a statistically significant reduction of phospholamban phosphorylation at Thr-17. No effects on Ca^{2+} transients and sparks were observed when activating Epac signaling in dox-treated mice, which showed a reduced expression of Epac proteins.

Our results indicate for the first time that Epac signaling is blunted after doxorubicin treatment and a decrease in phospholamban phosphorylation may underlie slower Ca^{2+} transients.

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Doxorubicin-induced cardiotoxicity: role of Epac signalling

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Doxorubicin (Dox), a widely used anticancer drug, can cause severe cardiac complications such as heart failure (HF). Among the pathophysiological mechanisms underlying dox-induced cardiotoxicity, remodelling of the β-adrenergic signalling appears to be involved but still need further investigation. Epac, an exchange protein directly activated by cyclic AMP, could play an important role in the progression of dox-induced HF in regard to the role of Rho-GTPases Rac1 and RhoA in dox side effects.

Therefore, we investigated the time- and dose-dependent effect of Dox both in vitro and in vivo on the transcriptional activity of cardiac remodelling and stress markers such as Atrial Natriuretic Factor (ANF), Serum Responsive Element-regulated c-fos (SRE) and muscle skeletal α-actin (SkM α-actin) and Epac's downstream effectors: Nuclear Factor of Activated T-cells (NFAT) and Myocyte Enhancer Factor-2 (MEF-2) using transient transfection and western blot.

In vitro, dox treatment (100nM) of neonatal rat cardiomyocytes for 24 hours resulted in an activation of ANF. Treatment with higher doses of dox leads to a decrease in the expression of remodelling markers. The transcription of MEF-2 is activated at 1 µM of doxorubicin but the transcriptional activity of NFAT decreases in a dose-dependent manner. In addition, we observed a strong reduction of Epac levels with doses as low as 10nM of Dox.

In vivo, echocardiography of mice treated with Dox by 3 iv injections reaching 12mg/kg cumulative dose showed a tendency to dilated cardiomyopathy. At the molecular level, we observed a time-dependent regulation of Epac1, Epac2 and RhoA in cardiotoxicity induced by Dox. Moreover, phospholamban phosphorylation at the CamKII site but not the PKA site was decreased in dox-treated mice at 15 weeks suggesting an alteration of SERCA2a activity specifically mediated by Epac1.

Our results indicate for the first time an integrated time-course of Dox induced-alteration in Epac signalling.

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Electrocardiographic findings in primary systemic amyloidosis, senile systemic amyloidosis and transthyretin-related amyloidosis

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Objective: This study sought to determine the spectrum of electrocardiographic (ECG) abnormalities found in patients with primary systemic amyloidosis (AL), senile systemic amyloidosis (SSA) and Transthyretin-Related Amyloidosis (TTR) and to evaluate the prognostic implications.

Methods: Between 2010 and 2012, 103 patients treated in the University Henri-Mondor Hospital with cardiac amyloidosis were included. 32 patients with AL, 63 patients with TTR and 11 patients with SSA. ECG were analysed regarding: rhythm, conduction, voltage, and ischaemic signs.

Results: The mean age and men prevalence were respectively 62±15 and 64%. Atrial fibrillation was significantly more frequent (p=0.005) in the SSA group (44%) compared to AL group (13%) and TTR group (7%). No significant difference was found between the three groups when low voltage was defined according to the Klein criteria or defined as a voltage inferior to 1 mV in all precordial limbs. But when it was defined as a voltage <0. 5 mV in all limb leads it was found significantly higher (p=0.006) in the AL population (36%) compared to the TTR (8%) and SSA (25%) groups. LV hypertrophy was rare in the three groups. No significant difference was found concerning conduction disorders (p=0.064) and the presence of Q waves (61% AL, 51% TTR, 33% SSA, p=0.51) or reduced R wave height (7% AL, 20% TTR, 0% SSA, p=0.14).

Conclusion: Electrocardiographic abnormalities are frequent in amyloidosis whatever the type.

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Postnatal overfeeding in mice increase susceptibility to cardiotoxicity and oxidative stress induced by doxorubicin

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Doxorubicin (DOX), a commonly used anticancer drug, is known to induce serious cardiotoxicity that is believed to be mediated in part by oxidative stress. Obese patients and experimental animals were shown to have increased susceptibility to DOX-induced cardiotoxicity. Postnatal overfeeding (OF) in rodents induces a permanent moderate increase in body weight in adulthood, associated with cardio-metabolic alterations. Our study investigated the influence of moderate overweight on the sensitivity to DOX-induced cardiotoxicity and oxidative stress.

Immediately after birth, litters of C57BL/6 mice were either maintained at 10 (normal-fed group, NF), or reduced to 3 in order to induce OF. At weaning, mice of both groups received a standard diet. At 4 months of age, mice were given a single intra-peritoneal injection of DOX (6 mg/kg). Cardiac function was followed using echocardiography. Myocardial mRNA expressions of antioxidant enzymes and of β-myosin heavy chain (β-MHC) were assessed 10 days after DOX injection. Oxidative stress was measured by electron spin resonance spectroscopy.

Four-months-old OF mice weighed 12% more than NF; nevertheless, their initial cardiac function was not modified. A single injection of DOX induced a significant decrease in left ventricular ejection fraction (LVEF) in both groups; however, the deterioration of cardiac function was higher in OF mice (49.9% vs 57%, $p<0.05$). In myocardial tissue, oxidative stress was increased (1.73±0.13 AU/mg vs 1.02±0.04 AU/mg, $p<0.01$), and the expression of Cu/Zn- superoxide dismutase and catalase mRNA were significantly decreased in OF mice ($p<0.05$). The expression of β-MHC in myocardial tissue was not significantly augmented in OF mice.

Altogether, these results suggest that postnatal overfeeding, despite inducing a very small increase in body weight in adulthood, can potentiate doxorubicin-induced cardiotoxicity and oxidative stress. The exact mechanism of this vulnerability needs now to be investigated.

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ORAI3 contributes to a new voltage independent calcium entry that increases in hypertrophic ventricular rat myocyte

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Increasing amount of data suggest the implication of STIM1, TRPCs and ORAI1 in mediating cardiac hypertrophy. In this work, we dissected the channel candidates accounting for the STIM1 dependent pro-hypertrophic sarcolemmal current previously depicted by our laboratory (Hulot et al. Circulation 2011). First we characterized the expression profile of ORAIs proteins in both normal and hypertrophied ventricular myocytes isolated from abdominal aortic banded rats. ORAI1-3 are equally expressed in control and hypertrophic cardiac myocytes. We used a non-viral method to deliver cy3-tagged siRNA to ventricular myocytes to knock-down the various channel candidates. The cardiac myocytes were isolated and the store-independent and store-dependent Ca^{2+} entry was measured on Fura-2 loaded cy3-labelled and control isolated cardiac myocytes. Whole cell patch-clamp technique was used to measure the currents. A mixture of ORAI1, 2 and 3 siRNAs completely inhibited both store-independent and store-dependent Ca^{2+} entry suggesting the implication of ORAIs. Surprisingly ORAI1 siRNA activated Ca^{2+} entry, but this knock-down was associated with an up-regulation of ORAI3. ORAI3 siRNA completely blocked store-independent Ca^{2+} entry. As a regulator of pro-hypertrophic Ca^{2+} signaling, ORAI3 may represent a novel drug and genetic target for modulating pathologic calcineurin/NFAT signaling in the heart and treating cardiac hypertrophy and heart failure.